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Comparison of Percentage Reduction of Total Petroleum Hydrocarbon on Treatment Using *Bryophylum pinnatum* Leaf: The Concept of Alcohol and Water as Solvent for Constituents Extraction

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Abstract

Investigation on the comparison of percentage reduction of total petroleum hydrocarbon on treatment using Bryophylum pinnatum leaf was indeed, considered by using the concept of alcohol and water as solvent for constituents' extraction. The extracts were introduced into the various bioreactors' set-up and the Total Petroleum Hydrocarbon (TPH) concentration was monitored with increase in contact period. The investigation revealed decrease in TPH concentration with increase in the percentage reduction of the TPH concentration. This behavior has demonstrated that the reaction process was not inhibited by any environmental factors and the biostimulant performance was rated good, because rapid growth of the microbes was experienced. The percentage reduction of the TPH concentration was high on ethanol-based extraction process compared to the water-based extraction process as well as more effective on loamy soil environment than clay soil environment. This research has shown the trend of the solvent extraction mechanism of ethanol and water in treatment of contaminated loamy and clay soil and the extent of remediation within the time period of 42 days.

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INTRODUCTION

The growth of human testicular cancer cells was inhibited by Isoalantolactone constituents. Human cervical cancer cells were examined *in vitro* using an electrolyte mobility shift assay linked to apoptosis-associated proteins. When it came to preventing cervical cancer, contaminants fared better than extracts [1, 2]. This plant is used to cure prostate cancer and the common cold, as well as to enhance breathing and overall wellness. The idea of preventing gentamycin-induced kidney damage was investigated in Wistar rats [3]. When it comes to treating hyperactive bladder, leaf juice is said to be more beneficial than anticholinergic medications [4]. The extracts were tested against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella aerogenes* [5].

Figure 1. Matured *Bryophylum pinnatum* plants.

Bacillus, P. aeruginosa, K. pneumoniae, S. typhi, E. coli, and S. aureus ATCC 13709 are common bacterial strains frequently used in antimicrobial susceptibility testing and microbiological research. This drug shields babies from placental and navel infections [6]. They have been shown to have antibacterial, antispasmodic, and anti-inflammatory properties [7, 8]. This extract may be useful in treating urolithiasis since it dramatically lowers urine oxalate levels. Kidney stones have long been treated with this plant. By converting calcium oxalate dehydrate crystals into monohydrates, *B. pinnatum* (Figure 1) dissolves kidney stones. Leaves' extracts lowered oxidative stress and kidney stone development [9, 10].

Permeable Reactive Barrier (PRB)

Because of its design and pollutant removal process, this technology is primarily thought of as a physical method for remediating contaminated groundwater. Researchers did note, however, that one of the various mechanisms (degradation, precipitation, and sorption) of pollutant removal in the PRB approach is biological reaction [11–13]. The role of microorganisms has been reported to be primarily as an enhancement rather than as an independent biotechnology, despite the fact that other terms, such as biological PRB, passive bio-reactive barrier, and bio-enhanced PRB, have been proposed to accommodate the bioremediation or biotechnology aspect of the technique [14]. Unless otherwise indicated, all variations of this technology, including the permeable reactive barrier itself, shall be referred to in this section as PRB. Generally speaking, PRB is an *in-situ* method for cleaning up groundwater that has been contaminated by various contaminants, such as heavy metals and chlorinated chemicals. This method involves immersing a permanent or semi-permanent reactive barrier (medium) in the path of contaminated groundwater, primarily composed of zero-valent iron [15].

Pollutants are trapped as they pass through the barrier under its natural gradient, where they undergo a series of processes that produce clean water [16–18]. The barriers should ideally be passive with minimal energy input, reactive enough to capture pollutants, permeable to let water through but not pollutants, affordable, easily accessible, and widely available [19]. The type of medium utilized, which is impacted by the type of pollution, hydrogeological and biogeochemical conditions, environmental and health effects, mechanical stability, and cost, is the primary determinant of this technique's success [20, 21]. Researchers have increasingly focused on merging PRB with other methods, such as electrokinetics, to address different types of pollutants [22–25]. When electrokinetic and PRB approaches were used, it was found that 90% of the nitrate in spiking clay soil was removed in 1 week [26]. Similarly, when electrokinetic soil flushing was combined with biological-PRB (Bio-PRB), after 2 weeks of operation, 30% of the diesel was eliminated from clay soil. Additionally, when Bio-PRB was combined with electrokinetics to treat diesel-polluted soils, there was a 39% decrease in diesel biodegradable components after 2 weeks [27–30].

It appears that these combined methods produced a distribution of surfactant biomass across the polluted soil and enabled it to maintain the proper environmental conditions (pH, temperature, and nutrients) required for microbial growth. It is interesting to note that in an artificial laboratory-scale aquifer, a white-rot fungus (*Trametes versicolor*) used as a bio-barrier caused approximately 97% of the Orange G dye to degrade, suggesting the fungus' potential for use as a barrier (PRB) in natural aquifers [31]. During the performance evaluation of PBR for remediation of dissolved chlorinated solvents in groundwater, the formation of carbonate precipitate in the iron zone was not found to be the main constraint on the observed performance; rather, a PRB was used to measure the precise velocity of the groundwater [32].

Maintaining the proper particle size distribution can help preserve the barrier permeability, which is essential for PRB success even while maintaining barrier reactivity is essential for PRB technique performance [33]. The PRB technique presents several operational challenges, including decreased long-term performance due to decreased barrier reactivity, loss of porosity, zero-valent iron (ZVI), and the inability to apply the technique to sites contaminated with certain chlorinated hydrocarbons and recalcitrant compounds. However, it was noted that the biodegradable polymer polyhydroxybutyrate (PHB) possesses a slow-release nutrition (carbon) capability that, when employed as a barrier, increased the elimination of chlorinated chemicals by promoting biological activity [34]. Reduced PRB efficiency may be the consequence of design errors and environmental variations that might make hydrogeological site assessment challenging [35]. Consequently, enhanced PRB designs and affordable advanced site characterization techniques will boost the technique's efficacy [36]. Additionally, some of the difficulties (loss of permeability under specific geological circumstances) related to the use of ZVI would be mitigated by the use of iron sulphide (FeS) barrier [37–40]. Furthermore, the extrapolation of PBR performance based on laboratory scale column experiments is likely to be impacted by model significant uncertainties; these uncertainties can be mitigated through independent experiments and field observation aimed at improving comprehension of the surface deactivation mechanism in iron PRBs [41]. Numerous descriptions of other designs, reactive media, benefits, drawbacks, and pollutants eliminated by the PRB approach have been published [42–45].

MATERIALS AND METHODS

Determination of Total Petroleum Hydrocarbon

Using a Gen Tech Master G with a split/split less injector, a J and W 30-m DB-5 column, and a FID detector, samples were examined in accordance with the USEPA 8015 procedure. The injector temperature was set to 240°C, the detector temperature was set to 300°C, and the carrier gas, helium, was set to 20 ml/min. It took 45 min to run the capillary column at 1 NI injection volume and a slow ramp rate of 40 to 310°C. Three working standards made from a stock solution of Accu standard 500 mg/ml in chloroform hydrocarbon window defining standard were used to calibrate the gas chromatograph (GC). For the analysis, a soil sample weighing roughly 5 g was used. Using 5 g of anhydrous sodium sulphate, the sample was chemically dried. Separate extraction was performed using 10 ml of dichloromethane, and o-terphenyl was employed as a stand-in. A glass wool/glass funnel was used to decant the extract after the extraction solvent and sample were shaken for 45 min using a vortex mixer. Before being sent to the GC for TPH analysis, the extract was given time to concentrate to 1 ml. The glass electrodes were standardized after every 10 determinations. The electrodes were dipped in distilled water when not in use, and the reference electrode was ensured to always contain saturated potassium chloride in contact with solid potassium chloride crystals. 3 to 4 drops of toluene were added in standard buffer solution to prevent growth of mold. The residual TPH percentage at any time was calculated using Eq. (1).

$$TPH_R(\%) = \frac{TPH_i - TPH_f}{TPH_i} \times 100\% \quad (1)$$

Where; TPH_R is the residual TPH percentage with time, TPH_i is the initial concentration of TPH and TPH_f is the concentration of TPH measured with time.

RESULTS AND DISCUSSION

Evaluation on Percentage TPH Reduction on Loam Soil Using Ethanol and Water Extracts

Figure 2 illustrates the trend of TPH percentage reduction on loamy soil when subjected to ethanol and water extracts of *Bryophylum pinnatum* leaves for treatment. The different mixed proportions of 100, 200, and 300 ml of the extracts monitored for 42 days decrease the concentration of hydrocarbon in clay and loamy soil except for the control without any treatment. The control option T1 had 11.25% reduction compared to the treated options; T3 had 57.89% when subjected to 100 ml water extract, T4 had 58% with 200 ml water extract, and T5 had 65.77% with water extract. In comparison with ethanol biostimulant of 100, 200, and 300 ml contained in T6, T7, and T8 had 77.78, 82.36 and 87.18% reduction respectively. The highest decrease in TPH was observed in T7 and T8 when subjected to ethanol extracts on loamy soil. The percentage of TPH degradation in Loamy soil increased in correspondence with an extension in the duration of remediation. However, the rate of TPH degradation also displayed a modest increase as the treatment weight was raised. Detailed results on TPH percentage reduction treatment on loamy soil using *Bryophylum pinnatum* leaves can be found in Appendix A.

Evaluation on Percentage TPH reduction on Clay Soil using Ethanol and Water

Figure 3 illustrates the trend of TPH percentage reduction on clay soil when subjected to ethanol and water extracts of *Bryophylum pinnatum* leaves for treatment. The different mixed proportion of 100, 200, and 300 ml of the extracts monitored for 42 days decrease the concentration of hydrocarbon in clay and loam soil except for the control without any treatment. The control option T2 had 9.05% reduction compared to the treated options; T9 had 54.97% when subjected to 100 ml water biostimulant, T10 had 60.81% with 200 ml water extract, and T11 had 62.63% with ethanol biostimulant. In comparison with water biostimulant of 100, 200, and 300 ml contained in T12, T13, and T14 had 54.97, 60.81 and 62.63% reduction respectively. Similar percentage of TPH reduction was experienced using mango leaves on silt loamy and clay soil for a treatment period of 28 days. They found out that 20 g of the sample had maximum degradation rate of 87.69% for clay soil, and for loam soil of 82.80%. In this study, highest decrease in TPH was observed in T10 and T11 when subjected to ethanol biostimulant on clay soil. The percentage of TPH degradation in clay soil increased in correspondence with an extension in the duration of remediation. However, the rate of TPH degradation revealed a modest increase in treatment weight on clay soil against the loam soil. Detailed results on TPH percentage reduction treatment on loam soil using *Bryophylum pinnatum* leaves can be found in Appendix A.

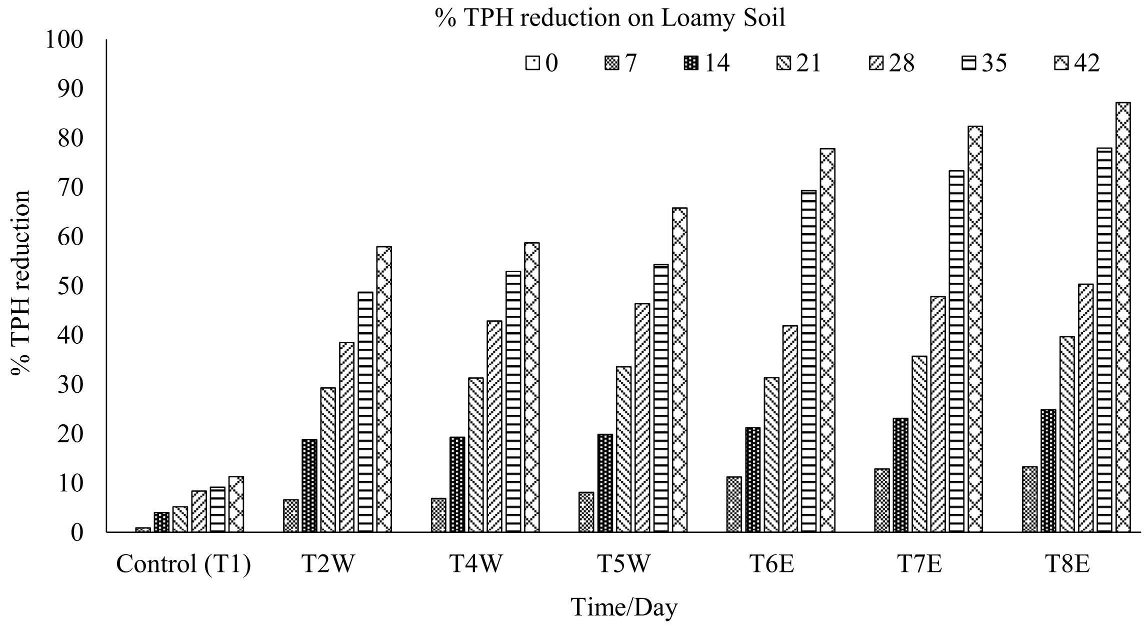

Figure 2. Reduction of TPH versus time on loamy soil treatment options.

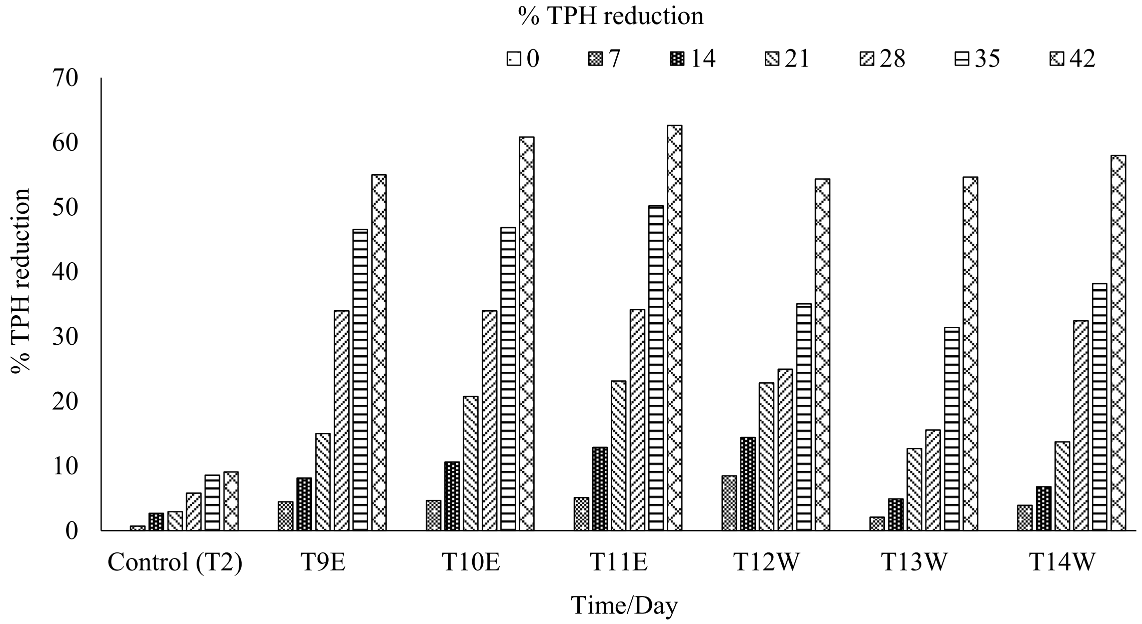

Figure 3. Reduction of TPH versus time on clay soil treatment options.

CONCLUSION

The conclusion of the findings is as stated:

1. The determination of the percentage reduction revealed the trend of bio-stimulant performance and its potential on treatment of contaminated site using solvent extraction.
2. Research has also revealed that remediation was more effective using alcohol extraction in both soil environments of loamy and clay.
3. Furthermore, the research demonstrated that TPH degradation was more on loamy soil environment compared to clay soil environment and this was attributed to the soil porosity.
4. Microbial growth was rapid at the progressive phase provided the process was inhibited.

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APPENDIX A

The comparison of percentage reduction of TPH on loamy soil treatment options and on clay soil treatment options is shown in Tables 1 and 2 respectively.

Table 1. Comparison of percentage reduction of TPH on loamy soil treatment options.

Time (days)	Control (T1)	T3W	T4W	T5W	T6E	T7E	T8E
7	0.885812473	6.57428379	6.8719	8.113554	11.21895	12.86698	13.32258
14	3.979736215	18.81032329	19.23927	19.82954	21.20879	23.06997	24.85127
21	5.148238418	29.27858548	31.28306	33.58012	31.32071	35.72606	39.64206
28	8.384185078	38.50029734	42.85682	46.36819	41.90085	47.79657	50.3349
35	9.127027254	48.69293663	52.91827	54.30313	69.25521	73.31608	77.92064
42	*11.24640986*	*57.89070291*	*58.70114*	*65.77623*	*77.77575*	*82.35986*	*87.17983*

Table 2. Comparison of percentage reduction of TPH on clay soil treatment options.

Time (days)	Control (T2)	T9E	T10E	T11E	T12W	T13W	T14W
7	0.7218	4.47668	4.67547	5.0825791	8.4857353	2.08671	3.89689
14	2.66597	8.11075	10.604	12.855813	14.400004	4.91782	6.78673
21	2.95112	14.9866	20.7218	23.142912	22.811183	12.6548	13.7292
28	5.78569	33.9381	33.9742	34.132443	24.938321	15.5290	32.4474
35	8.56766	46.5093	46.8227	50.212582	35.039206	31.3639	38.1797
42	*9.05294*	*54.9791*	*60.8152*	*62.631513*	*54.336803*	*54.6529*	*57.9747*

